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Acoustic Startle Response in Rats: Effect of Cat Odor and Social Context

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Abstract

The acoustic startle response is the reflexive response to an abrupt auditory stimulus. In this study it was quantified as maximum movement immediately subsequent to a loud tone. Startle response is enhanced by fear stimuli and is often used as a model for studying anxiety. Because anxiety levels are often dependent on context, the present study examined the effects of social peers and cat odor on startle reactivity. The presence of another rat did not alter basal startle reactivity, but introduction of another rat after shock significantly reduced the increase of startle reactivity that would normally have resulted from shock alone. This suggests an anxiolytic effect of social context in rats. Odor from cat fur was expected to enhance startle, as cats are natural predators of rats. Although there was a modest increase in startle in association with the cat odor, this effect did not reach significance.

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Approximately 19 million American adults suffer from anxiety disorders annually, such as panic disorder, post-traumatic stress disorder, generalized anxiety disorder, and social phobias (National Institute of Mental Health, 1994). These debilitating disorders are augmented by a considerable amount of research that seeks more effective models and treatments. One model for experimentation that has to do with anxiety is the acoustic startle response paradigm.

The acoustic startle response is a reflexive response to an abrupt auditory stimulus. This is a cross-species measure and can be found in humans as well as other animals. It is typically enhanced by fear and anxiety and has often been used as a metric of these states. This reflex is organized predominantly in the brainstem, but these circuits are also modulated by higher neural systems associated with fear (Davis, 1998). Some of these circuits may include the basal forebrain cholinergic pathways in top-down processing (Sarter, Givens, & Bruno, 2001) and ascending pathways through the amygdala and basal forebrain (Berntson, Sarter, & Cacioppo, 2003).

The present study was designed to evaluate the effects of social context on startle reactivity. Two social contests were examined, the presence of a predator odor and the presence of a conspecific.

Rats are thought to be predisposed to show an aversion to certain odors in cat fur. Increased anxiety responses measured by elevated plus maze, the hole board, and the general activity test have been observed (Adamec & Shallow, 1993, and McGregor, Schrama, Ambermoon, & Dielenberg, 2002). Cat odor from cat fur was expected to

potentiate startle response by inducing innate fear-related reactions to predator cues. If such were the case, it would offer a naturalistic model of anxiety.

Looking more closely at effects of odor has merit, especially because the sensitive olfactory systems of rats project directly to the amygdala, which has been implicated in emotional response (Richardson & McNally, 2003). Furthermore, the use of naturalistic cat odor might access fear circuits differently than conventional methods associated with shock and other aversive stimuli. For example, the fox odor extract TMT was shown to differentially activate dopamine systems in the amygdala and nucleus accumbens as well as endocrinological systems as compared to conditioned fear stimuli (Morrow, Redmond, Roth, & Ellsworth, 2000). Evidence also suggests that cat odor selectively activates Fos expression the medial amygdala, ventromedial and dorsomedial hypothalamus, dorsal premammilary nucleus, and the periaqueductal gray (Dielenberg, Hunt, & McGregor, 2001 and Dielenberg & McGregor, 2001).

Because anxiety levels are often dependent on social context, the second portion of this study was designed to investigate startle reactivity in the presence of another rat. Rats are highly social organisms, but can also be competitive. To explore this issue, trials with social peers were compared to control trials, trials with a foot shock, and trials with a foot shock and a peer.

The use of new contexts may broaden the scope in which results can be appropriately applied, especially in human behavior relating to situations and disorders involving anxiety and fear. Expansion of the paradigms used to assess anxiety will create a wider diversity of knowledge and will allow for specialized focus on subsets of anxiety disorders. Overall, studies of this type may help to increase our understanding of human

behavior by creating animal models and may improve our capacity to effectively support those afflicted with anxiety disorders.

Experiment 1

Method

Subjects. The subjects were 19 naive adult male Sprague-Dawley rats, 90-120 days old and weighing 350-420 g at the beginning of testing. They were separately housed in a vivarium at 73°F with 45% humidity and a 12:12 light/dark cycle that began the light portion at 6:00 a.m. and began the dark portion at 6:00 p.m. The rats had unrestricted access to food and water.

Apparatus. Rats were placed in a clear acrylic cylinder with slots that allow air flow and adequate space to turn around. The cylinder was positioned upon an accelerometer which contains an electric grid capable of quantifying the rat's horizontal and vertical movement (San Diego Instruments, San Diego, CA), with extra weight on the vertical coefficient. The startle chamber was contained within a large sound attenuated chamber. A Super-Tweeter (Radio Shack, Fort Worth, TX) and a RCA Mini-Speaker (PRO-X44AV RCA, Indianapolis, IN) were placed near the accelerometer and played startle tones at 95 dB and white noise at 55 dB, respectively. A microphone was also kept within the testing chamber to verify that the tones were being played and as a place marker to identify when to calculate the startle response. Data from the accelerometer and microphone were collected by Acknowledge Software (Biopac, Goleta, CA). Cat odor was kept in a bottle topped with gauze and sealed with a lid between usages. An empty bottle topped with gauze and sealed between use was the control.

Procedure. The cat odor portion of the study consisted of two within-group conditions, cat odor present and cat odor absent, counterbalanced over two days to minimize order effects. Each rat participated in one condition per day. Each day of the week prior to testing, the rats were weighed, handled for five minutes, acclimatized to the startle chamber for five minutes between 12:30 p.m. and 2:00 p.m., and given a cereal treat afterwards. During the first two days of acclimatization each rat was placed in the startle chamber with no other stimulus. Over the subsequent three days and during startle testing, continuous white noise was played at 55 dB. Rats were placed in the acrylic cylinder within the startle chamber for a five minute waiting period, followed by ten .5 s tones at 95 dB separated by 12 s inter-tone intervals, and then ten more tones of the same volume, duration, and temporal spacing from which baseline data were obtained. Directly after baseline data were acquired, the door to the sound attenuated chamber was opened and either a bottle of cat odor covered by gauze or an empty bottle similarly covered was placed next to the accelerometer. The door was closed and the session continued with a two minute waiting period, followed by a sample of ten tones identical to baseline tones, a four minute waiting period, and a second sample of ten tones.

Data Analysis. Startle reactivity was assessed using Acknowledge software and quantified as the apex in activity measured by the accelerometer within 100 ms of onset of the startle tone. Test responses were calculated as the percent change of the startle amplitude during experimental conditions compared to pre-test baseline values. Once percent change was determined for each trial, an average across rats was computed for each trial type, cat odor present trials and also in cat odor absent trials.

Results and Discussion

Data taken 2 minutes and 8 minutes post-stimulus were similar and were collapsed into one set of post stimulus scores. Mean startle reactivity increased from baseline by 77.84% (*SD* = 156.48%) in cat odor trials and by 33.77% (*SD* = 107.20%) in control trials (See Figure 1). Two animals were not included in statistical analysis because of insufficient data. This repeated-measures design was evaluated using a one-tailed *t*-test which revealed that the difference was not significant, $t(16) = 1.43$, $p > .05$. Also, no order effects were observed.

Although the statistics did not support the hypothesis that cat odor would potentiate the startle response in rats, the startle was about twice as large when cat odor was presented. This might reflect insufficient statistical power to detect the difference, given the variance and present number of rats. Other laboratories have found variable evidence for and against an effect of cat odor to increase startle reactivity (Morrow, Redmond, Roth, & Ellsworth, 2000). This finding will require follow-up studies to explore the potential differences in methodology that might account for inconsistent results.

Experiment 2

Method

Subjects. The experimental subjects were 13 adult male Sprague-Dawley rats that participated in the cat hair portion of this study. The six remaining rats from the previous experiment were used as peers in this portion of the study. The rats were 90-120 days old, weighing 350-420 g, and housed in the same conditions previously described with ad libitum access to food and water.

Apparatus. The apparatus used in this portion of the study was the same as in Experiment 1 except for the addition of shocking equipment and a cage to contain the peer. The shocking device was the barred floor of an operant conditioning box that was 10x5x7 inches. The operant box was sealed in a sound attenuated chamber. The house light and fan within the chamber were kept off. The cage for the peer was made of wire with a wire top. It was situated near, but not contiguous with, the accelerometer in the sound attenuated cabinet.

Procedure. The day succeeding completion of the Experiment 1, rats began the acclimation period for the next portion of the study. For three days prior to testing, each rat was weighed, handled for five minutes, acclimatized, and given a treat afterwards. They were acclimated in the same manner as in the previous experiment except that after spending five minutes in the startle chamber with 55dB white noise they were placed in a separate operant box which contained the shock apparatus for two minutes and then placed back in the startle chamber for another three minutes.

This portion of the study consisted of four within-group conditions counterbalanced with the Latin Square technique over four days in such a way that no two rats proceeded through conditions in the same sequence, to prevent order effects. These four conditions consisted of: no shock/peer (NS/P), no shock/no peer (NS/NP), shock/peer (S/P), and shock/no peer (S/NP). Each rat participated in one condition per day.

The procedure followed that of the first experiment closely. The main differences were the introduction of shock and/or peer in place of cat fur. After baseline was acquired, the rat was removed from the cylinder and placed in the shock chamber. It

remained there for two minutes and at the end of this period it received a shock on two days and no shock on the other two. In conditions with a social peer, during this two minute period the peer rat was placed in a cage within the startle chamber adjacent to the accelerometer. No peer was introduced to any particular experimental rat more than once. The experimental rat was then placed back into the startle chamber and the experiment continued as did Experiment 1 after introduction of stimulus.

Data Analysis. Movements were quantified and compared with the same method as before. Test responses were calculated by percent difference from baseline values.

Results and Discussion.

NS/P trials on average showed a 15.84% increase in mean startle reactivity as compared to baseline, NS/NP reflected a 1.26% increase, S/P revealed a -21.06% decrease from baseline values, and S/NP showed a 97.15% increase (See Figure 2). Comparisons were drawn across the four categories of this 2 x 2 factorial design by using an ANOVA. One subject was not included because the peer escaped and possibly confounded the measurer by climbing on top of the accelerometer. The ANOVA results reveal no overall main effect for shock, $F(1,11) = 3.05$, $p > .05$, or peer, $F(1,11) = 3.95$, $p > .05$. A significant interaction was found where presence of a peer reduced the effect of shock, $F(1,11) = 6.69$, $p < .05$. A *t*-test reveals that there was in fact a significant effect of shock, $t(11) = 2.47$, $p < .05$, where shock elevated startle reactivity.

The finding of this portion of the study is consistent with other the literature. In two reviews of social influences on stress, stress reduction is found to be facilitated by social affiliations (DeVries, 2002 and DeVries, Glasper, & Detillion, 2003). The reviews emphasize the role of blunted hypothalamic-pituitary-adrenal (HPA) activity and the

neuropeptide oxytocin as a possible modulator. Many of these examples involve bonded pairs, potential mating partners, monogamous species, or animals that show different glucocorticoid circadian rhythms and different control of HPA axis from rats. Although the examples given may not be directly applicable to the present study, they do seem to lend support for the socially facilitated reduction in acoustic startle response due to shock in this study and point to an intriguing possible mechanism, suppression of HPA axis.

In conjunction with these theories of social buffering, a study conducted by Wilson (2001) demonstrated that the release of prolactin (a hormone associated with stress) in rats during open field exposure was attenuated when another rat was present, but only when they were able to touch. Another study that supported social stress buffering, demonstrated that presence of a conspecific reduced stress-induced hyperthermia, behavioral responses to stress, and Fos expression. The authors also showed that the state of the accompanying rat could affect the outcome, where shocked partners did not reduce stress levels as much as nonshocked conspecifics (Kiyokawa, Kikusui, Takeuchi, & Mori, 2004).

Taken together, these studies are compatible with the results of the present experiment, and support the role of socially facilitated stress reduction in rats.

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Figure Captions

Figure 1. Mean percentage difference from baseline of cat odor as compared to control. Although cat odor increased from baseline more than control, this difference did not reach significance.

Figure 2. Mean percentage difference from baseline of each trial type: no shock/peer (NS/P), no shock/no peer (NS/NP), shock/peer (S/P), and shock/no peer (S/NP). There was an interaction between shock and peer such that the elevated startle reactivity due to shock was reduced by presence of a peer.

Figure 1.

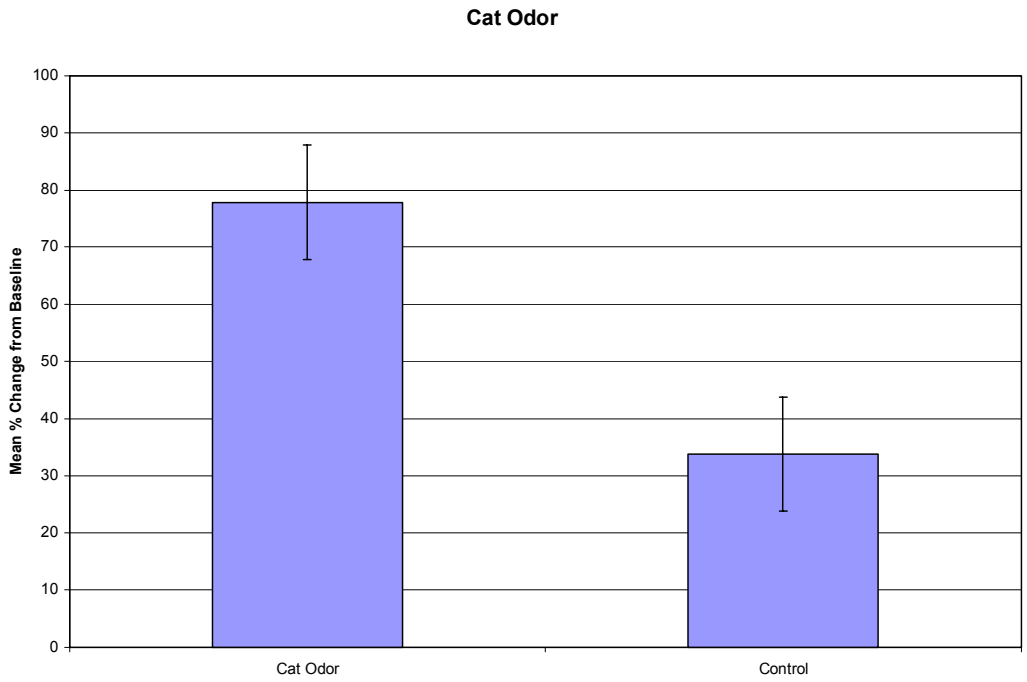

Figure 2.